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Report

Vav1 as a Central Regulator of Invadopodia Assembly

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Summary

Invadopodia are protrusive structures used by tumor cells for degradation of the extracellular matrix to promote invasion [1]. Invadopodia formation and function are regulated by cytoskeletal-remodeling pathways and the oncogenic kinase Src. The guanine nucleotide exchange factor Vav1, which is an activator of Rho family GTPases, is ectopically expressed in many pancreatic cancers, where it promotes tumor cell survival and migration [2, 3]. We have now determined that Vav1 is also a potent regulator of matrix degradation by pancreatic tumor cells as depletion of Vav1 by siRNA-mediated knockdown inhibits the formation of invadopodia. This requires the exchange function of Vav1 toward the GTPase Cdc42, which is required for invadopodia assembly [4, 5]. In addition, we have determined that Src-mediated phosphorylation and activation of Vav1 are both required for, and, unexpectedly, sufficient for, invadopodia formation. Expression of Vav1 Y174F, which mimics its activated state, is a potent inducer of invadopodia formation through Cdc42, even in the absence of Src activation and phosphorylation of other Src substrates, such as cortactin. Thus, these data identify a novel mechanism by which Vav1 can enhance the tumorigenicity and invasive potential of cancer cells. These data suggest that Vav1 promotes the matrix-degrading processes underlying tumor cell migration and further, under conditions of ectopic Vav1 expression, that Vav1 is a central regulator and major driver of invasive matrix remodeling by pancreatic tumor cells.

Results and Discussion

Vav1 Expression Promotes Degradation of the Extracellular Matrix

Ectopic expression of Vav1 in pancreatic cancers leads to increased tumor cell survival, enhanced cell migration, and a poor prognosis [2, 3]. Accordingly, RNAi-mediated depletion of Vav1 in DanG pancreatic adenocarcinoma cells inhibited transwell invasion (Figure 1A). In addition to upregulating migratory signaling pathways, tumor cells degrade and remodel the extracellular matrix (ECM) to allow for escape from the primary tumor and metastasis. The actin cytoskeletal changes required for migration and invasion are regulated by Rho guanosine triphosphatases (GTPases), including Rac1, RhoA, and Cdc42, the activation of which is controlled by guanine nucleotide exchange factors (GEFs) [6, 7]. As Vav1 is a GEF and activator of Rho GTPases, and is ectopically expressed in tumor cells, we hypothesized that Vav1 could promote the invasive process of matrix degradation (Figure S1A available online) [7]. To test this, DanG cells were depleted of Vav1 using siRNA, then plated on fluorescent gelatin-coated coverslips for 7 hr. Control transfected cells showed robust matrix degradation (Figure 1B). However, Vav1 depletion, confirmed by western blot analysis (Figure 1C), reduced both the number of cells competent to degrade matrix (Figure 1D) and the area of degradation per cell (Figure 1E). Similar results were observed in three other Vav1-expressing pancreatic tumor cell lines (CFPAC, Panc04.03, and HPAF-II; Figures S1B–S1E). Thus, in addition to regulating tumor cell survival and migration, Vav1 also promotes degradation of the ECM by pancreatic cancer cells.

Tumor cells degrade the ECM through invadopodia, invasive protrusions that are sites for targeted secretion of metalloproteases [8]. Invadopodia consist of an actin core and require the activity of Cdc42 for the actin nucleation and polymerization necessary for their formation. We hypothesized that Vav1 was required for either the formation or the maturation of invadopodia. To test this, DanG cells were depleted of Vav1 by siRNA, plated on fluorescent gelatin, and then stained for actin or cortactin. Control cells formed numerous puncta, which stained positive for both actin and cortactin, and often coincided with sites of matrix degradation, indicating the presence of functional invadopodia. However, depletion of Vav1 dramatically reduced the number of actin puncta (Figure 1F). These data suggest that when Vav1 is ectopically expressed in pancreatic tumor cells, it promotes ECM degradation through the formation of invadopodia.

Vav1 Regulates Invadopodia Formation and Matrix Degradation through Cdc42

While Vav1 is an exchange factor for Rho family GTPases, it does have GEF-independent adaptor functions [9, 10]. Thus, we tested if matrix degradation required Vav1 GEF activity. Re-expression of wild-type (WT) Vav1 completely rescued matrix degradation in the Vav1 knockdown cells (Figures 1G and 1H). However, GEF-inactive Vav1 L278Q was unable to restore matrix degradation, demonstrating that the GEF activity of Vav1 is required and suggesting that Vav1 regulates ECM degradation through its action as an exchange factor for Rho GTPases.

Vav1 is primarily a GEF for Rac1, though it also has exchange activity toward Cdc42 and RhoA [11, 12]. Therefore, we sought to determine which Rho GTPase mediated matrix degradation downstream of Vav1 in pancreatic cancer cells. Expression of a constitutively active form of Rac1, Cdc42, or RhoA should overcome the requirement for a GEF and restore matrix degradation in cells depleted of Vav1. Vav1-depleted DanG cells were transfected with empty vector or active Rac1 (Q61L), RhoA (Q63L), or Cdc42 (Q61L). Only expression of active Cdc42, but not Rac1 or RhoA, restored matrix degradation in Vav1 knockdown cells, both in the percentage of cells degrading matrix and in the area of degradation per cell, suggesting that Cdc42 activation is downstream of Vav1 (Figures 2A–2C). Even though Vav1 regulates Rac1 activity, matrix degradation did not require Rac1, as knockdown of Rac1 did not affect the ability of Cdc42 to rescue matrix degradation in Vav1-depleted cells (Figure S2A).

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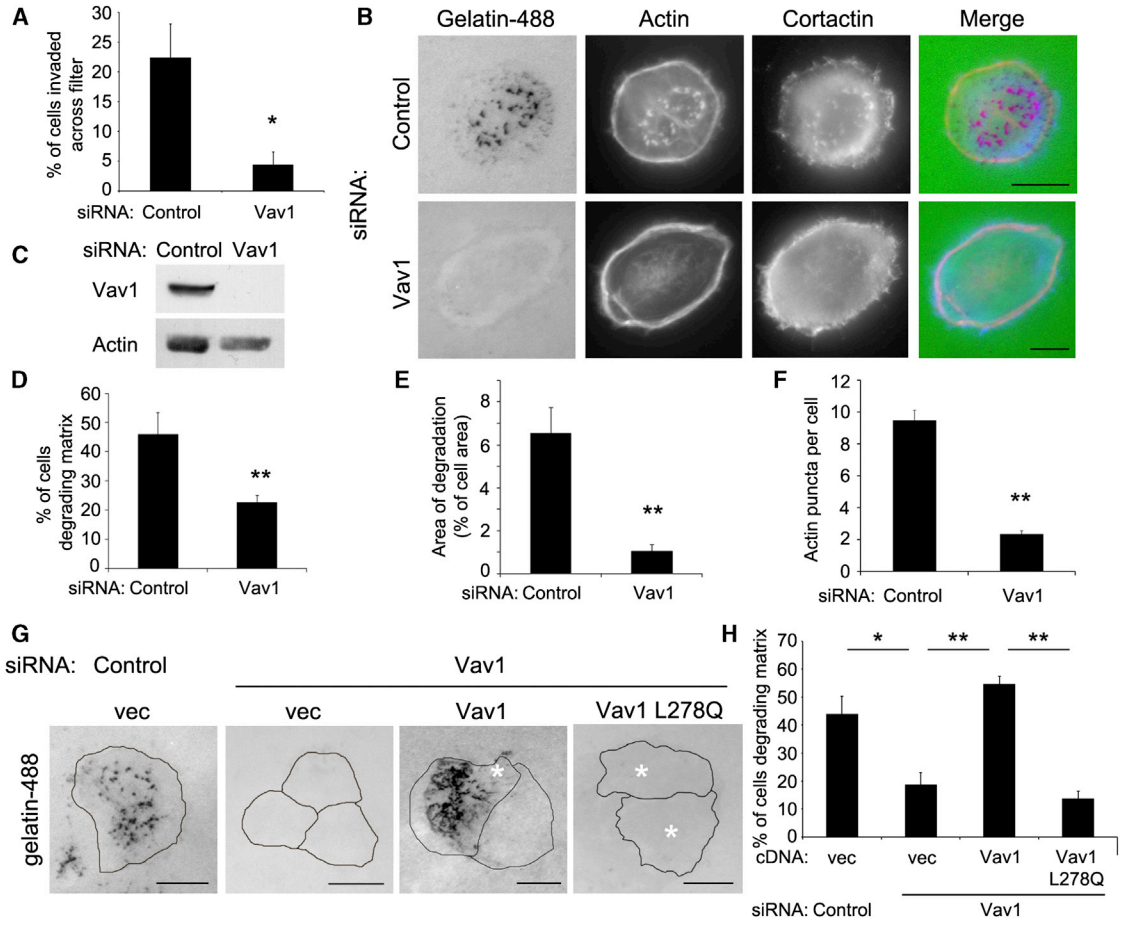

Figure 1. Vav1 Expression Promotes Matrix Degradation by Pancreatic Tumor Cells

(A) Vav1 is required for invasive migration of pancreatic tumor cells. DanG cells were transfected with siRNA against Vav1 or a control siRNA and plated in a transwell invasion assay. The percentage of cells invaded across the filter after 72 hr was measured.

(B) Depletion of Vav1 using siRNA inhibits degradation of a gelatin matrix. DanG cells were transfected with siRNA against Vav1 or a control siRNA and then plated on fluorescent gelatin for 7 hr. Cells were stained with TRITC-Phalloidin and cortactin to detect the actin cytoskeleton and invadopodial puncta. While control cells degraded substantial amounts of matrix, the cells treated to reduce Vav1 levels did not.

(C) Parallel samples from (B) were lysed and immunoblotted to confirm protein knockdown.

(D) The percentage of cells degrading the gelatin matrix was scored. At least 100 cells were scored per condition.

(E) The area of degradation was quantified in at least 20 cells per condition.

(F) The number of actin puncta per cell was scored (n > 100 cells per condition).

(G) The GEF activity of Vav1 is required for matrix degradation. DanG cells were depleted of Vav1 using siRNA and then transfected with WT Vav1 or GEF-inactive Vav1 (L278Q). Cells were plated on fluorescent gelatin for 7 hr, stained for ectopic Vav1 expression (not shown), and then scored for matrix degradation. Re-expression of WT Vav1, but not GEF-inactive Vav1, restored matrix degradation. Asterisks indicate transfected cells. vec, empty vector.

(H) The percentage of cells degrading the gelatin matrix was scored (n > 100 cells per condition).

All graphed data indicate the mean ± SEM of at least three independent experiments. *$p < 0.05$; **$p < 0.01$. Scale bars represent 10 μm. See also Figure S1.

Further, Vav1 mediates invadopodia formation through Cdc42, as expression of constitutively active Cdc42 also restored the presence of actin puncta in Vav1 knockdown cells (Figures 2D and S2B). Consistent with this observation, expression of dominant-negative Cdc42 (T17N) or siRNA-mediated knockdown of Cdc42 inhibited matrix degradation (Figures 2E and S2C) [5]. These data suggest that Vav1-mediated Cdc42 activation promotes invadopodia function and matrix degradation.

Further, RNAi-mediated depletion of Vav1 in DanG cells reduced Cdc42 activation by 60% in pancreatic cancer cells, as measured by a biochemical pull-down for active Cdc42 (Figure 2F). In addition, overexpression of WT Vav1, but not GEF-inactive Vav1 L278Q, caused a 2-fold increase in Cdc42 activation in PANC1 pancreatic cancer cells (which do not express endogenous Vav1, Figure 2G). Taken together, these data indicate that Vav1 regulates matrix degradation in pancreatic cancer cells through activation of Cdc42.

Vav1 Mediates the Effects of Src on Matrix Degradation

The tyrosine kinase Src is aberrantly activated in many cancers and is a potent inducer of invadopodia formation and matrix degradation [13–15]. Src family kinases phosphorylate multiple substrates involved in invadopodial structure and dynamics, including neural Wiskott-Aldrich syndrome protein (N-WASP), cortactin, the scaffold Tyrosine kinase substrate 5 (Tks5), and the phosphatidylinositol 5-phosphatase synaptojanin 2 (PI) [15–20]. Vav1 is a substrate for Src family kinases in hematopoietic cells, where phosphorylation of critical residues (Tyr142, Tyr160, and Tyr174) induces a conformational

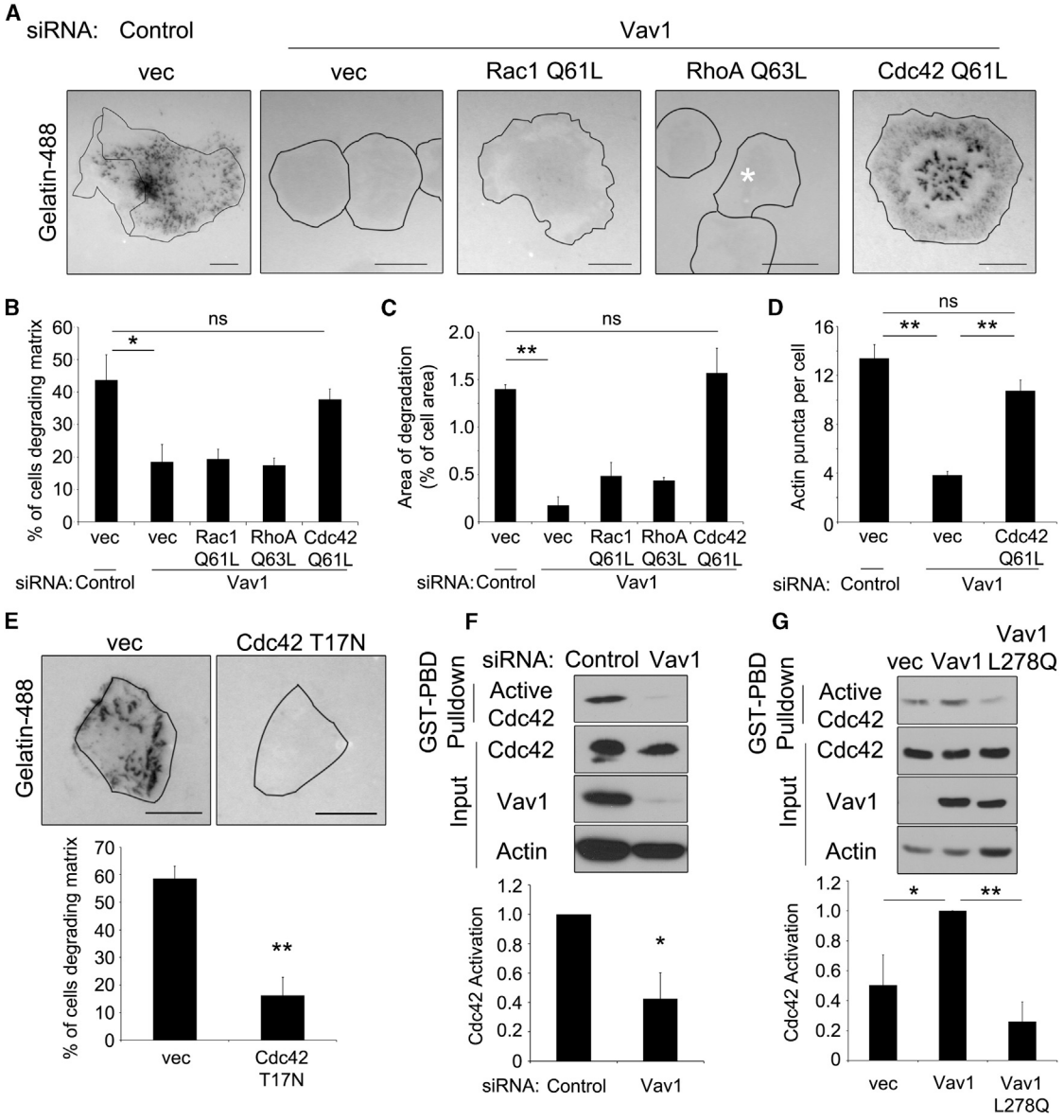

Figure 2. Vav1 Regulates Matrix Degradation through Cdc42

(A) Constitutively active Cdc42, but not constitutively active Rac1 or RhoA, rescues matrix degradation in Vav1 knockdown cells. DanG cells were depleted of Vav1 by siRNA and then transfected with myc-tagged Rac1 (Q61L), RhoA (Q63L), or Cdc42 (Q61L). Cells were plated on a fluorescent gelatin matrix for 7 hr and then fixed and stained for the myc epitope tag (not shown).

(B and C) The percentage of cells degrading the gelatin matrix was scored (n > 100 cells per condition) (B), and the area of degradation was quantified (n > 20 cells per condition) (C).

(D) Cells transfected as described above were stained for actin and the myc epitope tag (not shown), and the number of actin puncta per cell was scored (n > 40 cells per condition).

(E) Dominant-negative Cdc42 inhibits matrix degradation in Vav1-expressing cells. DanG cells were transfected with empty vector or Cdc42 T17N, and matrix degradation was assessed as described. Cdc42 T17N expression was verified by immunofluorescence for Cdc42 (not shown). The percentage of cells degrading matrix was scored in at least 50 cells per condition.

(F) Cdc42 activation is impaired in the absence of Vav1. DanG cells were depleted of Vav1 by siRNA and subjected to a glutathione S-transferase (GST)-p21-activated kinase binding domain (PBD) pull-down for active Cdc42.

(G) Ectopic Vav1 expression enhances Cdc42 activation. PANC1 cells were transfected with empty vector, WT Vav1, or GEF-inactive Vav1 (L278Q) and were analyzed by GST-PBD pull-down for active Cdc42.

For both (F) and (G), levels of active Cdc42 were normalized to total Cdc42.

All graphed data indicate the mean ± SEM of at least three independent experiments. *$p < 0.05$; **$p < 0.01$; ns, no statistically significant difference. Scale bars represent 10 μm. See also Figure S2.

change to allow activation of Vav1 [21–24]. Mutation of Vav1 to mimic its activated phosphorylated state increases Rac1 activation and migration in breast cancer cells [25]. Therefore, we hypothesized that Src signals through Vav1 in pancreatic tumor cells to modulate invadopodia formation and matrix degradation. Vav1 is phosphorylated downstream of Src in

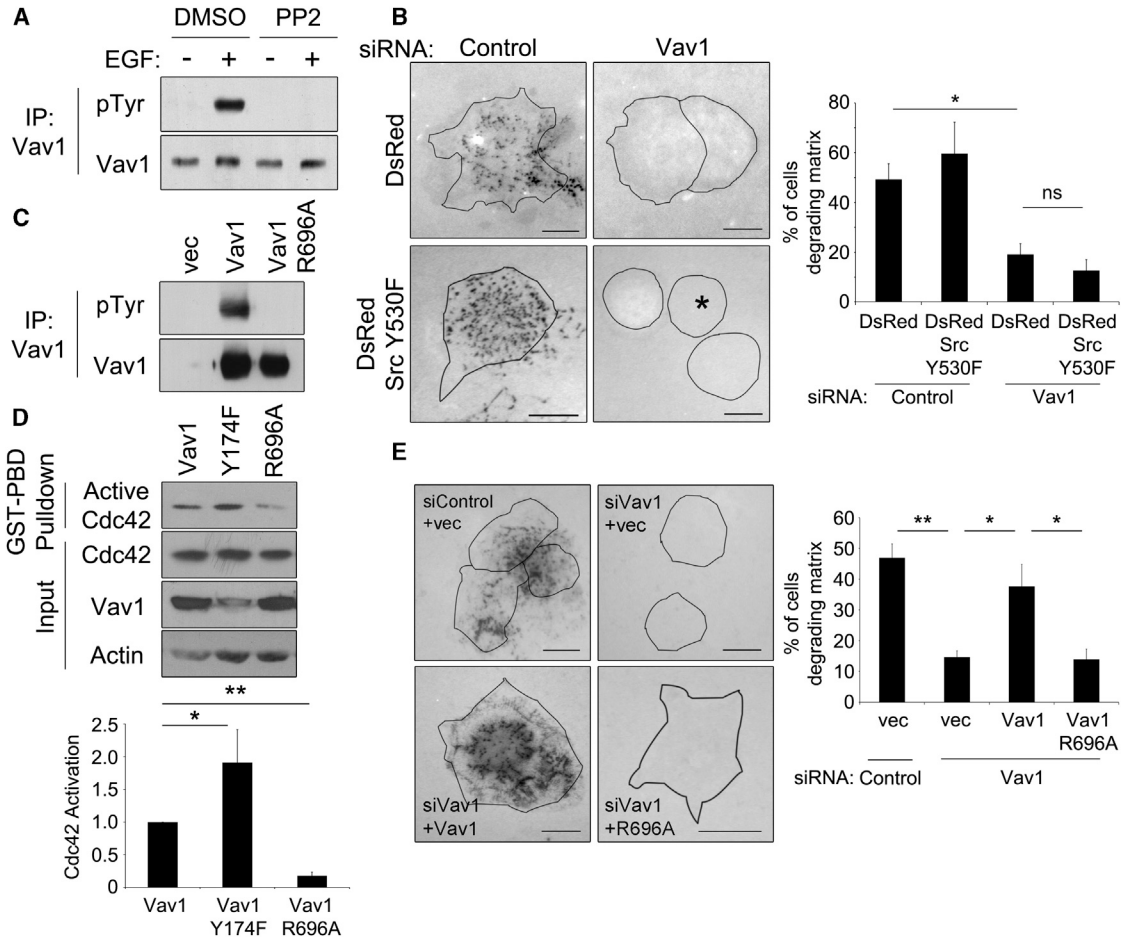

Figure 3. Src-Mediated Activation of Vav1 Is a Central Regulator of Matrix Degradation by Pancreatic Tumor Cells

(A) Vav1 is phosphorylated downstream of Src family kinases in pancreatic tumor cells. DanG cells were treated with PP2 (25 μM) for 4 hr, then stimulated with EGF (100 ng/ml) for 5 min. Lysates were immunoprecipitated (IP) for Vav1 and immunoblotted for phosphotyrosine. PP2 treatment abolished Vav1 tyrosine phosphorylation.

(B) Vav1 is required for Src-mediated matrix degradation. DanG cells depleted of Vav1 were transfected with dsRed vector or dsRed-tagged active Src (Y530F) and plated on fluorescent gelatin for 7 hr. The asterisk indicates a transfected cell. The percentage of cells degrading matrix was scored in >50 cells per condition. Even in the presence of active Src, cells depleted of Vav1 do not degrade the gelatin matrix.

(C) The mutation R696A blocks Vav1 phosphorylation. PANC1 cells were transfected with control vector, WT Vav1, or Vav1 R696A, stimulated with EGF, and processed as described in (A). Tyrosine phosphorylation of Vav1 was severely inhibited by the R696A mutation.

(D) Vav1 phosphorylation and activation regulate Cdc42. PANC1 cells were transfected with WT Vav1, active Vav1 (Y174F), or Vav1 R696A and subjected to a GST-PBD pull-down. Active Cdc42 was normalized to total Cdc42 and compared to cells expressing WT Vav1.

(E) Vav1 activation is required for matrix degradation. DanG cells were depleted of Vav1 by siRNA, then transfected to express either WT Vav1 or Vav1 R696A and plated on fluorescent gelatin for 7 hr. The percentage of cells capable of degrading the matrix was scored in >50 cells per condition. WT Vav1 rescued matrix degradation, but not Vav1 R696A.

All graphed data represent the mean ± SEM of three independent experiments. *$p < 0.05$; **$p < 0.01$; ns, no statistically significant difference. Scale bars represent 10 μm. See also Figure S3.

DanG cells, as epidermal growth factor (EGF)-stimulated tyrosine phosphorylation of Vav1 was inhibited by the Src inhibitors PP2 or dasatinib (Figures 3A and S3A). Consistently, Vav1 tyrosine phosphorylation was increased 2-fold by expression of active Src Y530F, but not inactive Src Y419F (Figure S3B). Together, these data suggest that Src family kinases promote Vav1 tyrosine phosphorylation and, therefore, Vav1 activation.

To test if Vav1 is required for Src-mediated matrix remodeling, DanG cells were transfected with constitutively active Src (Y530F) and then depleted of Vav1 by siRNA. Remarkably, matrix degradation in the Src-expressing cells was almost completely ablated in the absence of Vav1 (Figure 3B), suggesting that Vav1 functions downstream of Src to support matrix remodeling. As DanG cells have high levels of active Src and matrix degradation, additional expression of active Src did not increase matrix remodeling. Significantly, in Panc04.03 cells, which have lower levels of endogenous matrix degradation, expression of active Src Y530F caused a 3-fold increase in matrix degradation, which was completely blocked by knockdown of Vav1 (Figure S3C). Together, these data indicate a requirement for Vav1 in Src-mediated matrix degradation.

We next tested if Src-mediated phosphorylation and activation of Vav1 were required for matrix degradation by preventing Vav1 phosphorylation. Paradoxically, structural studies have determined that the Y174F mutation induces the open, "active" conformation, even in the absence of a phosphate, inducing constitutive activation [26, 27]. Therefore, an indirect,

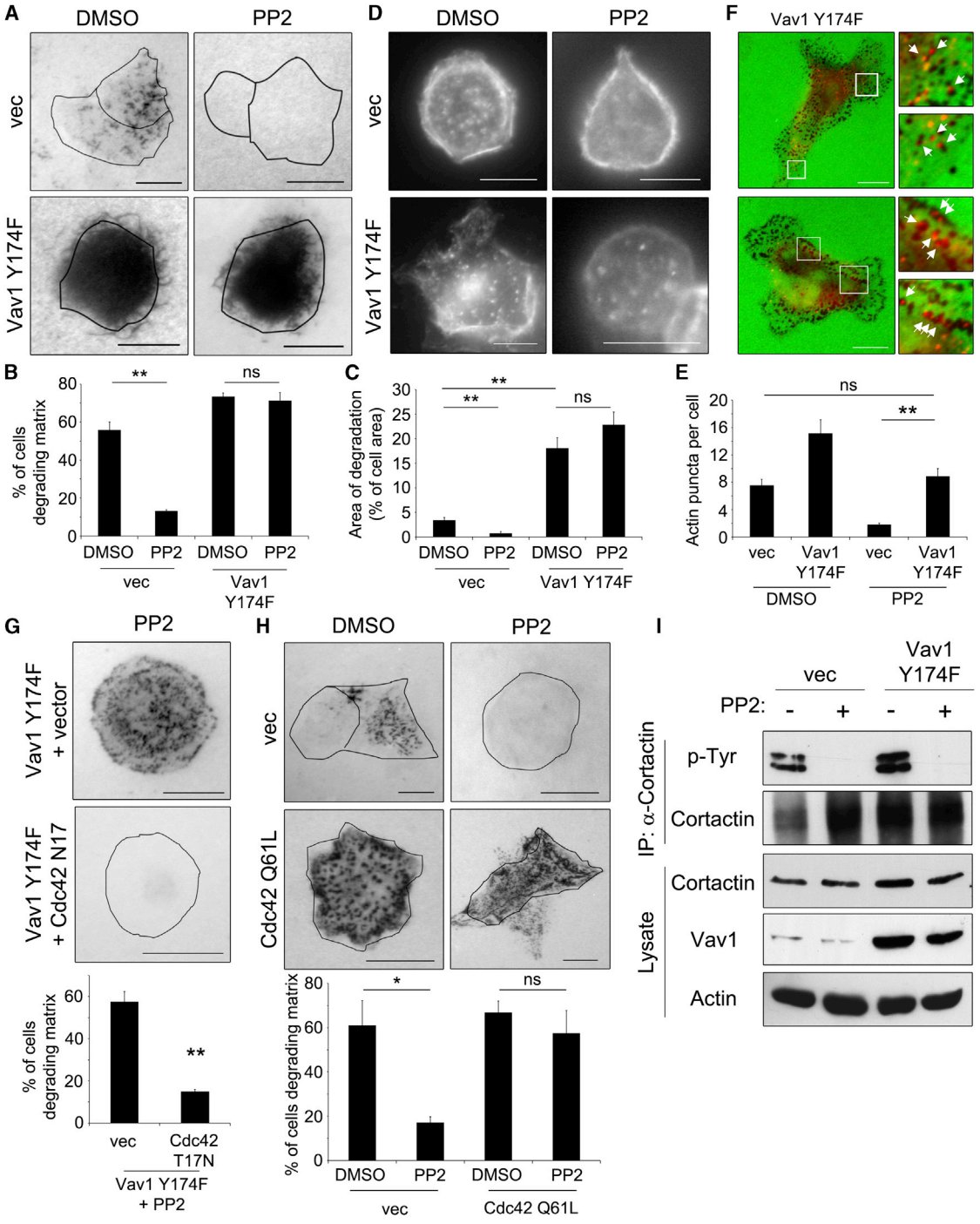

Figure 4. Vav1 Activation Is Sufficient for Matrix Degradation Downstream of Src

(A) DanG cells were transfected with control vector or active Vav1 Y174F, then treated with the Src inhibitor PP2 (10 μM) or DMSO vehicle control while plated on a fluorescent gelatin matrix for 7 hr.

(B) The percentage of cells degrading the matrix was scored (n > 100 cells per condition).

(C) The area of degradation was quantified in >20 cells per condition. Note that Vav1 Y174F potently induced matrix degradation, even in the presence of PP2.

(D) Vav1 Y174F rescues the formation of invadopodia. DanG cells were transfected as described in (A) and plated on fluorescent gelatin overnight in the presence of BB94 (1 μM). The BB94 was washed out, and cells were incubated for 4 hr with or without PP2 prior to staining for actin to mark invadopodia and Vav1 to detect transfected cells (not shown).

(E) The number of actin puncta per cell was scored in at least 20 cells per condition.

(F) Vav1 Y174F can be detected at sites of matrix degradation. DanG cells were transfected with Vav1 Y174F and plated on fluorescent gelatin (green) for 7 hr. Cells were fixed and stained for Vav1 (red). The boxed regions are magnified at right. Arrows indicate Vav1 Y174F puncta that colocalize with sites of matrix degradation.

(legend continued on next page)

inactive Vav1 form was used by mutating the Src homology 2 (SH2) domain of Vav1 (R696A), which impairs its ability to interact with tyrosine-phosphorylated substrates required for Vav1 recruitment and activation by Src [28]. Consistent with a defect in recruitment and activation, Vav1 R696A is not tyrosine phosphorylated in EGF-stimulated cells (Figure 3C). Further, Vav1 phosphorylation and activation correspond with an ability to activate Cdc42. PANC1 cells transfected to express WT Vav1, active Vav1 Y174F, or phosphorylation-deficient Vav1 R696A showed marked differences in Cdc42 activation. Cells expressing phosphorylation-defective, inactive R696A Vav1 exhibited much less activation than cells expressing WT Vav1 (Figure 3D), whereas active Vav1 Y174F induced a 2-fold increase in Cdc42 activation compared to WT Vav1. These data suggest that Vav1 phosphorylation and activation downstream of Src family kinases regulate Cdc42 activation in pancreatic cancer cells.

We then tested if phosphorylation of Vav1 controls matrix degradation. Notably, the defect in matrix degradation induced by Vav1 knockdown was completely rescued by re-expression of WT Vav1, but not the R696A mutant (Figure 3E), suggesting that Vav1 recruitment and phosphorylation are required for Cdc42-mediated degradation of the ECM.

Vav1-Cdc42 Activation Is Sufficient for Matrix Degradation Downstream of Src

From these findings, we hypothesized that Vav1 phosphorylation alone could be sufficient to induce invadopodia formation and matrix degradation downstream of Src. Thus, expression of an active Vav1 could potentially drive matrix degradation, even when Src activity is ablated by a Src inhibitor. The Src inhibitor PP2 blocked matrix degradation in control cells (Figures 4A–4C). While expression of the active Vav1 Y174F markedly enhanced matrix degradation in control cells, most remarkable was that PP2-treated cells exhibited the same massive increase in degradation. Similar results were observed in the presence of the Src inhibitors SU6656 and dasatinib, and in CFPAC cells (Figures S4A–S4C). Similarly, PP2 treatment inhibited the formation of invadopodia, and this defect was rescued by expression of Vav1 Y174F (Figures 4D and 4E). Thus, even in the presence of a Src inhibitor, activation of Vav1 induces degradation of the matrix, suggesting that Vav1 activation is sufficient for invadopodial matrix degradation downstream of Src.

Consistent with these findings, active Vav1 Y174F showed increased localization to sites of matrix degradation and to actin puncta marking invadopodia compared to cells expressing WT Vav1. Importantly, the Src inhibitory drug PP2 did not reduce this colocalization (Figures 4F and S4D). We speculate that Vav1 localization is transient or occurs only at early stages of invadopodia formation. These stages may then be enhanced by the constitutive activation of Vav1 using the Y174F mutation, thereby facilitating Vav1 detection at these sites. The localization of Vav1 Y174F to sites of matrix degradation supports the premise that Vav1 is a component of invadopodial assembly, and further, our data suggest that Vav1 activation is dominant to upstream Src-mediated signaling pathways.

We sought to confirm that this Src-independent degradation was in fact mediated through Vav1-induced Cdc42 activation, as shown above (Figure 2). Expression of dominant-negative Cdc42 (T17N) or siRNA-mediated knockdown of Cdc42 markedly reduced the degradation induced by Vav1 Y174F in PP2-treated DanG cells (Figures 4G and S4E). Taken together, these data indicate that Cdc42 is necessary downstream of active Vav1 to promote matrix degradation downstream of Src.

As an extension of these findings, we predicted that Vav1 Y174F should promote Cdc42 activation, even in the presence of the Src inhibitor. While this activation was observed, we also found an unexpected activation of Cdc42 following PP2 treatment in both control cells and cells expressing Vav1 Y174F. Even though Vav1 potently regulates Cdc42 activation (Figure 2), this suggests that Cdc42 activity could also be regulated by other Src-dependent factors as Vav1 would be inactive in these cells. Further, as Src inhibitors clearly reduce invadopodia formation (Figure 4), this finding suggests that Cdc42 activation, while necessary, may not be sufficient for matrix degradation downstream of Src. As an alternative approach to this question, we tested if constitutively active Cdc42 (Q61L) was sufficient to restore matrix degradation even upon inhibition of Src. As observed previously, PP2 treatment inhibited matrix degradation by DanG cells, and notably, Cdc42 Q61L was sufficient to overcome the loss of Src activity and restore matrix degradation, similar to expression of Vav1 Y174F (Figure 4H). These data suggest that hyperactivation of Cdc42 via mutation or expression of the mutant GEF is sufficient for matrix degradation downstream of Src. While we cannot exclude that Vav1 and Cdc42 may act in parallel pathways, our data do support the model that Src-mediated activation of Vav1 drives Cdc42 activity, which is then sufficient for invadopodia formation and matrix degradation. Cdc42 promotes invadopodia formation by signaling through N-WASP and Arp2/3 to induce actin nucleation and branching [29, 30]. It remains to be determined if Cdc42 promotes matrix degradation solely through the defined N-WASP-Arp2/3 pathway or if other downstream effectors are also required.

Unexpectedly, this finding suggests that phosphorylation of other Src substrates may not be required for invadopodia formation. Therefore, we wanted to test the phosphorylation of cortactin, a known substrate downstream of Src via Arg/Abl involved in invadopodia formation [31], in Vav1 Y174F-expressing cells treated with a Src inhibitor. If cortactin was not phosphorylated, it would suggest that active Vav1 could induce invadopodia-mediated matrix degradation even in

(G) DanG cells virally transduced to stably express Vav1 Y174F were transfected with empty vector or dominant-negative Cdc42 (T17N, myc tagged), then plated on fluorescent gelatin in the presence of 10 μM PP2. Overexpressed Vav1 and Cdc42 T17N were identified by immunofluorescence for Vav1 and myc, respectively (not shown). Cdc42 T17N inhibits matrix degradation induced by Vav1 Y174F.

(H) DanG cells were transfected with myc-tagged active Cdc42 (Q61L) or empty vector and plated on fluorescent gelatin with or without PP2 (10 μM) for 7 hr. Cdc42 Q61L expression was verified by anti-myc immunofluorescence (not shown). Cdc42 Q61L was sufficient to rescue matrix degradation in the presence of PP2. For (E) and (F), the percentage of cells degrading matrix was scored in at least 50 cells per experiment.

(I) The Src substrate cortactin is not phosphorylated upon PP2 treatment. DanG cells stably expressing GFP vector or Vav1 Y174F were treated with PP2 (10 μM) or DMSO control, then immunoprecipitated for cortactin and blotted for phosphotyrosine. Note that cortactin is not phosphorylated in the PP2-treated cells, even in the cells expressing Vav1 Y174F.

All graphed data represent the mean ± SEM of three independent experiments. *$p < 0.05$; **$p < 0.01$; ns, no statistically significant difference. Scale bars represent 10 μm. See also Figure S4.

the absence of cortactin phosphorylation. In control cells, PP2 treatment blocked tyrosine phosphorylation of cortactin, confirming that it is phosphorylated downstream of Src (Figure 4I). Importantly, cortactin phosphorylation was unaffected by the Vav1 Y174F mutation. Cortactin phosphorylation was blocked by PP2 treatment even in cells expressing Vav1 Y174F, which form invadopodia and potently degrade the matrix. These data demonstrate that active Vav1 drives invadopodia-mediated matrix degradation, even in the absence of Src activation and phosphorylation of a known substrate. Further, the data suggest that Src phosphorylation of cortactin may be dispensable for matrix degradation in tumor cells with activated Vav1. Perhaps Cdc42-driven N-WASP activation is sufficient, and further enhancement regulated by cortactin phosphorylation is not necessary. Alternatively, a recent report described phosphorylation-independent interactions between cortactin and SH2 domains of its binding partners, suggesting that cortactin phosphorylation may not be required for its effects [32].

These data strongly support a central role for Vav1 in promoting invadopodia and matrix degradation. Pancreatic cancer cell lines that express Vav1 have become dependent upon it as knockdown of Vav1 inhibits matrix degradation, even in the presence of multiple other Cdc42 GEFs. However, while Vav1 is ectopically expressed in many pancreatic cancers, it is not expressed in all tumor cells (Figure S1B), some of which are still able to form invadopodia and degrade matrix. We hypothesize that Vav1-negative tumors and tumor cell lines have upregulated the invadopodial and invasive machinery by other mechanisms, such as a different Cdc42 GEF. Accordingly, we screened a panel of nine pancreatic cancer cell lines for the expression of six different Cdc42 GEFs, and consistent with our prediction, nearly every tumor cell line exhibited a different pattern of GEF expression (Figure S4F). These data reflect the heterogeneity of tumors among individuals and strengthen the case for an individualized approach to cancer treatment. We hypothesize that differential expression of GEFs is important for defining the pathway regulating invadopodia formation in specific cell types. It will be key to determine how/why different GEFs are utilized (either independently or in combination) and how converging signals may activate one or more GEFs selectively.

Activating mutations in Vav1 have not been described in pancreatic cancers. However, pathways that lead to Vav1 phosphorylation and activation are known to be hyperactivated, including EGF receptor and Src [13]. Therefore, it is highly likely that in tumors where Vav1 is ectopically expressed, its activation is upregulated due to hyperactivation of regulatory signaling pathways. Thus, activation of any pathway leading to activation of Src family kinases or, ultimately, Vav1, could potently upregulate the invasive machinery. In addition, while some of the Src inhibitors used in this study have additional kinase targets, this only strengthens the importance of active Vav1 and describes it as a more global regulator of matrix degradation downstream of multiple signaling pathways.

We have recently reported that Vav1 is required for Rac1-mediated formation of lamellipodia and subsequent migration of tumor cells [3]. In addition, Vav1 is a potent regulator of transendothelial migration of leukocytes and also contributes to CXCL12-induced MT1-matrix metalloprotease (MMP) expression and invasion by melanoma cells [33, 34]. The current findings further implicate Vav1 in the processes of invasion and migration, namely through the formation of invadopodia and matrix degradation. This process requires Vav1 activation of Cdc42, demonstrating that in pancreatic tumor cells, ectopically expressed Vav1 can signal through multiple pathways. This is consistent with a previous report that Vav1-induced oncogenic transformation requires multiple signaling pathways, including Rac1, Cdc42, and RhoA, as well as nuclear factor κB (NF-κB) and Jun-N-terminal kinase (JNK) [35]. Indeed, these data implicate Vav1 in multiple stages of the tumorigenic and metastatic program and suggest that upon its aberrant expression, Vav1 is a pivotal signaling node in pancreatic cancers.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.11.013.

Acknowledgments

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